PATENT SPECIFICATION

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COMPLETE SPECIFICATION

NO DRAWINGS

New Tetracycline Derivatives

We, CARLO ERBA S.p.A., an Italian Body Corporate, of Via Imbonati 24, Milan, Italy, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to tetracycline derivatives.

It is known that tetracycline salts are not stable in aqueous solutions having a nearly neutral pH. They tend to form precipitates and their pharmacological application is therefore difficult.

The present invention provides soluble tetracycline derivatives which avoid this inconvenience and have a practically neutral reaction.

The tetracycline derivatives of the present invention are made by condensing together tetracycline, oxytetracycline, chlortetracycline, demethyltetracycline, or demethylchlortetracycline, with formaldehyde and an amide of an α-amino acid having the following structure:

$$R—CH(NH_2)—CONR^1R^2$$

where R is hydrogen, alkyl or substituted alkyl such that the compound $RCH(NH_2)COOH$ is a known, naturally-occurring amino-acid and R^1 and R^2 may be the same or different and represent hydrogen, alkyl, hydroxyalkyl, aminoalkyl, alkylaminoalkyl, or dialkylaminoalkyl or R^1 is hydrogen and R^2 is amino. Preferably R is such that the amino acid

$$R—CH(NH_2)—COOH$$

is glycine, alanine, serine or lysine. The preferred amides are the unsubstituted amides and the *N*-(β-hydroxyethyl)- and *N,N*-di(β-hydroxyethyl)-amides. The lower alkyl substituted amides, such as the mono- or dimethyl or ethyl amides are also useful. The term "lower alkyl" is used herein to refer to alkyl of up to six carbon atoms.

The tetracycline derivatives of the invention are Mannich-type condensation products having the formula:

$$T—CH_2—NH—CH(R)—CO—NR^1R^2$$

where T is a radical derived from tetracycline, oxytetracycline, chlortetracycline, demethyltetracycline, or demethylchlortetracycline by removal of a hydrogen atom, believed to be that *ortho* to the phenolic hydroxyl group, and R, R^1 and R^2 are as defined above, and obtainable by the condensation of a said tetracycline with formaldehyde and an α-aminoacid amide by the process defined above. In any case the analysis of the compounds of the invention indicates that their molecules consist of a radical derived from the tetracycline and a radical derived from the amide joined by a methylene group (derived from the formaldehyde).

The condensation of the tetracycline, formaldehyde and α-amino acid amide is conveniently carried out in an inert solvent, e.g. methanol, dioxane, or dimethylformamide, at room or slightly elevated temperature. The formaldehyde used can be gaseous, in aqueous solution (e.g. formalin), or in the form of the trimer (trioxane).

The following Examples illustrate the invention.

EXAMPLE I

0.6 cc. Formaldehyde (38% aqueous solution) and 0.6 g. l-alaninamide are added to a solution of 3 g. of tetracycline free base in 120 cc. methanol. A clear solution is obtained and allowed to stand for 2 hours. It is then evaporated to a small volume and diluted with diethyl ether. A precipitate forms and is filtered off and dried *in vacuo* at 50°C. The product thus obtained is a yellow powder melting at 150-156°C.

[Price 4s. 6d.]

Analysis:
Calc. for $C_{25}H_{28}N_4O_9$:
C 57.34% H 5.92% N 10.29% O 26.44%.
Found:
C 56.85% H 5.71% N 10.25% O 26.84%.

Similar soluble compounds can be obtained by following the procedure of this Example but employing; in place of tetracycline itself, chlortetracycline, oxytetracycline, demethyltetracycline, or demethylchlortetracycline; and, in place of alaninamide, glycinamide hydrochloride, l-lysinamide, serinamide, monomethylamide glycine, or diethylamide glycine.

The product obtained by reacting tetracycline with formaldehyde and glycinamide hydrochloride has a melting point of 148-152°C. The corresponding compounds when l-lysinamide, serinamide, and the monomethylamide of glycine are used have melting points of 145-150°C., 160-163°C., and 134-138°C. respectively.

EXAMPLE II

0.6 cc. Formaldehyde solution and 1.8 g. of the β-hydroxyethylamide of d,l-α-alanine are added to a solution of 6 g. of tetracycline in 200 cc. dimethylformamide. The solution is kept at 40°C. for 2 hours, and then concentrated to a small volume under vacuum and diluted with diethyl ether. The precipitate which forms is filtered off and dried *in vacuo* at 50°C. The product thus obtained is a yellow powder melting at 130-140°C.

Analysis:
Calc. for $C_{28}H_{33}N_5O_{10}$:
C 57.13% H 6.16% N 9.51% O 27.18%.
Found:
C 55.15% H 6.12% N 8.88% O 27.49%.

Similar products can be obtained using β-hydroxyethylamide-glycine, when the product has m.p. 135-138°C., β-hydroxyethylamide l-lysine, when the product has m.p. 130-135°C., or β-hydroxyethylamide d-serine in place of the β-hydroxyethylamide of d,l-α-alanine.

EXAMPLE III

1.1 cc. Formaldehyde (38% aqueous solution) and 1.8 g. of the diethanolamide of glycine are added to a solution of 5 g. tetracycline in 150 cc. dioxane. The solution is kept at room temperature for 2 hours and is then evaporated to a small volume. Tetracyclinemethylenediethanolamide glycine precipitates on adding diethyl ether.

Similarly can be obtained: tetracycline-methylene-diethanolamide d,l-alanine; tetracycline-methylene-diethanolamide l-lysine; and tetracycline-methylene-diethanolamide serine.

The invention provides also pharmaceutical compositions comprising one or more of the new tetracycline derivatives in association with a pharmaceutical carrier. Such compositions are preferably made up in a form suitable for oral or parenteral administration.

For oral administration the new compounds can be mixed with conventional diluents and tabletting materials and made up into tablets, pills and powders (which may be encapsulated). Alternatively the new compounds can be incorporated in a conventional syrup base.

For parenteral administration (for which they are especially suited), the new compounds may be dissolved in water, or another known injectable medium such as physiological saline, and the compositions sterilized, and filled into ampoules for storage before use.

WHAT WE CLAIM IS:—

1. Process for the preparation of water-soluble derivatives of a tetracycline which comprises reacting tetracycline, oxytetracycline, chlortetracycline, demethyltetracycline, or demethylchlortetracycline, with formaldehyde, and an α-amino-acid amide of the formula:

$$R—CH(NH_2)—CO—NR^1R^2$$

where R is hydrogen, alkyl or substituted alkyl such that the compound $RCH(NH_2)COOH$ is a known, naturally occuring amino acid and R^1 and R^2 may be the same or different and represent hydrogen, alkyl, hydroxyalkyl, aminoalkyl, alkylaminoalkyl, or dialkylaminoalkyl or R^1 is hydrogen and R^2 is amino.

2. Process according to claim 1 in which R is such that the amino-acid

$$RCH(NH_2)COOH$$

is glycine, alanine, serine or lysine.

3. Process according to claim 1 in which tetracycline, oxytetracycline, chlortetracycline, demethyltetracycline, or demethylchlortetracycline is reacted with formaldehyde and either the amide, or the *N*-β-hydroxyethylamide, or the *N,N*-di(β-hydroxyethyl)amide of glycine, alanine, serine, or lysine.

4. Process according to any of claims 1 to 3 in which the reaction is carried out in an inert solvent.

5. Process according to claim 1 substantially as described in any of Examples I to III.

6. Water-soluble tetracycline derivatives of the formula:

$$T—CH_2—NH—CH(R)—CO—NR^1R^2$$

where T is a radical derived from tetracycline, oxytetracycline, chlortetracycline, demethyltetracycline, or demethylchlortetracycline by removal of a hydrogen atom and R, R^1 and R^2 are as defined in claim 1, and obtainable by the condensation of a said tetracycline with formaldehyde and an α-amino acid amide in accordance with the process of any one of claims 1-5.

7. Tetracycline derivatives as claimed in claim 6 in which the residue R is such that

the acid

$$R—CH(NH_2)—COOH$$

is alanine, glycine, lysine or serine.

8. Tetracycline derivatives as claimed in claim 6 or 7 in which R^1 and R^2 are each hydrogen or β-hydroxyethyl.

9. A water-soluble tetracycline derivative as claimed in claim 6 substantially as described in any of the foregoing Examples.

10. A water-soluble tetracycline derivative obtained by the process of any one of claims 1 to 5.

11. A pharmaceutical composition comprising one or more of the compounds claimed in any of claims 6-10 in association with a pharmaceutical carrier.

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